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McGREGOR & ADLER, LLP

P.O. BOX 710509
HOUSTON, TEXAS 77271-0509

WRITER'S DIRECT DIAL
(713)-777-2321
WRITER'S DIRECT FACS.
(713)-777-6908
E. MAIL: BAADLER@flash.net

INTELLECTUAL PROPERTY LAW
(PATENT, BIOTECHNOLOGY, COMPUTER,
TRADEMARK & TRADE SECRET LAW)

February 10, 2000

Docket No.: D6017CIP

The Assistant Commissioner Of Patents And Trademarks
BOX PATENT APPLICATION
Washington, DC 20231

Dear Sir:

Transmitted herewith for filing is the Continuation-in-part patent application, which claims benefit of priority under 35 U.S.C. 120 of non-provisional application U.S. Serial No. 09/112,874 filed July 9, 1998, in the:

Name of: **Sanders, et al.**
For: **Targeted Destruction of Pests**

CLAIMS AS FILED

<u>Fee for:</u>	<u>Small entity</u>	<u>Amount</u>
Basic fee	\$345	\$345
Each independent claim in excess of 3 (2)	\$39	\$78
Each claim excess of 20 (0)		
Multiple dependent claim		

TOTAL FILING FEE \$423

___ Please charge my Deposit Account No. _____ in the total amount of the filing fee and the assignment recordation fee if any.

X A check in the amount of **\$423** to cover the filing fee is enclosed.

X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 07-1185.

X Any additional fees under 37 CFR 1.16.

X Any application processing fees under 37 CFR 1.17.

X Small Entity Statement

A small entity statement is enclosed and its benefit under 37 CFR 1.28(a) is hereby claimed.

X Relate Back--35 U.S.C. 119(e)

This Continuation-in-part application claims benefit of priority under 35 U.S.C. 120 of non-provisional Serial No. 09/112,874 filed July 9, 1998.

Assignment

The application is assigned by the inventors to the _____.

Sequence Listing

The sequence listing is enclosed, including a paper copy, a copy on disk for the computer readable form and a compliance letter indicating that the sequence listing on the paper copy and the disk are one and the same.

X Power of Attorney

X is attached.

X Address all future communications to:

Benjamin Aaron Adler
McGREGOR & ADLER, LLP.
8011 Candle Lane,
Houston TX 77071
(713) 777-2321

X Two photocopies of this sheet are enclosed.

Date: _____

Feb 10, 2000



Benjamin Aaron Adler, Ph.D., J.D.
Counsel for Applicant
Registration No. 35,423

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Sanders et al.

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GROUP ART UNIT:

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FILED: February 10, 2000

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EXAMINER:

SERIAL NO.:

§

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FOR: Targeted Destruction of Pests

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DOCKET: D6017CIP

Commissioner of Patents and Trademarks

BOX PATENT APPLICATION

Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 CFR 1.10

Dear Sir:

I hereby certify that the following documents, which are attached, are being deposited, under 37 CFR 1.10, with the United States Postal Service "Express Mail Post Office to Addressee" service as Express Mail No. EL516784358US in an envelope addressed to: Commissioner of Patents and Trademarks, Box Patent Application, Washington, D.C. 20231, on the date indicated below:

- 1) Continuation-in-part patent application + 4 sheets of drawings;
- 2) Two (2) Combined Declarations and Powers of Attorney;
- 3) Verified Statement of Small Entity Status;
- 4) Transmittal Letter; and;
- 5) A check for Filing fee \$423 and return postcard.

Please return the enclosed postcard acknowledging receipt of this correspondence.

Respectfully submitted,

Date:

Feb 10, 2000
McGREGOR & ADLER, LLP
8011 Candle Lane
Houston, Texas 77071
(713) 777-2321
BAADLER@flash.net

Benjamin Aaron Adler
Benjamin Aaron Adler, Ph.D., J.D.
Registration No. 35,423
Counsel for Applicant

Applicant or Patentee: Sanders et.al. Attorney's
Serial or Patent No.: _____ Docket No.: 6017CIP
Filed or Issued:
For: Targeted Destruction of Pests

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 CFR 1.9(f) and 1.27(c)) - NONPROFIT ORGANIZATION

I hereby declare that I am an official of the nonprofit organization empowered to act on behalf of the concern identified below:

NAME OF ORGANIZATION Research Development Foundation
ADDRESS OF CONCERN 402 North Division Street
Carson City Nevada 89703

TYPE OF ORGANIZATION:

X TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) AND 501(c)(3))

I hereby declare that the above identified nonprofit organization qualifies as a nonprofit organization as defined in 37 CFR 1.9(e), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization identified above with regard to the invention, entitled As above by inventor(s) described in ☒ the specification filed herewith
☐ application serial no. _____, filed
☐ patent no. _____, issued

If the rights held by the above identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING JAMES F. WEILER

TITLE OF PERSON OTHER THAN OWNER VICE PRESIDENT

ADDRESS OF PERSON SIGNING ONE RIVERWAY SUITE 1560

SIGNATURE

DATE 2/10/2010

TARGETED DESTRUCTION OF PESTS

5

BACKGROUND OF THE INVENTION

10

Cross-Reference to Related Application

This application is a continuation in part of U.S. application Serial No. 09/112,874, filed July 9, 1998 which claims benefit of provisional application U.S. Serial No. 60/052,132, filed July 10, 1997, now abandoned.

15

Field of the Invention

The present invention relates generally to immunology and genetic engineering technology. Specifically, the present invention relates to immunological engineering to produce novel reagents that target poisons to antigens such as cell surface

20

molecules on the cells of microvilli in the midgut of imported fire ant queens and other pests.

Description of the Related Art

5 Imported fire ants are an ecological and financial disaster in Texas as well as other states in the Southern United States. Imported fire ants were accidentally introduced into the U.S. in the 1930s. These pests completely upset and destroy natural ecosystems, and have detrimental economic effects in agriculture
10 (large mounds damage machinery), ranching (loss of newborn livestock), and recreation and tourism (loss of game birds and rendering park and resort areas uncomfortable at best).

A specific problem of fire ant control is how one should control or eliminate imported insect species without destroying
15 native insect species. This problem pertains to numerous non-native animal species that have been introduced in all parts of the United States. Imported species often have a competitive advantage over native species since, in many cases, they have developed enhanced reproductive strategies and do not have natural predators in their
20 new environment (1). Thus, it is important to eliminate the foreign

species. On the other hand, the native species should not be eliminated, as the proper balance of a particular ecosystem includes the presence of that native species.

Presently, the art includes the various methods of fire ant control. Chemical poisons, such as AMDRO are well known in the art and are used frequently. Such poisons, however, may pollute the environment, and indiscriminately eliminate native species as well as foreign species.

Thus, the prior art is deficient in an pest eradication product which is environmentally sound and specifically targets and eliminates only pests such as imported fire ants. The present invention fulfills this long-standing need and desire in the art.

15

SUMMARY OF THE INVENTION

The present invention is drawn to a safe, cost-effective, environmentally-friendly and ecologically-sound bioengineered product for managing pests such as imported fire ants, and a method of making this product. Immunological and genetic engineering

techniques are used to generate monoclonal antibodies (mAbs) and resulting Fab fragments as well as viruses (phage) that display antibody fragments, called single heavy or light chain V-gene fragments (scFv) or displayed heavy and light chain V-region fragments (Fab) which exhibit high-avidity specific binding to cells of the pest such as the microvilli of the midgut of imported fire ant queens.

For targeted delivery and destruction of specific pests, but not native species, the specific monoclonal antibodies and phage displayed scFv and Fab fragments are conjugated, e.g., chemically, using established procedures, to toxins such as gelonin, bacterial endotoxins, or other toxins. Furthermore, bispecific Fab's or scFv, with one arm of the Fab exhibiting specificity to the targeted cell membrane extracellular domain, and the other arm of the Fab exhibiting specificity to gelonin, bacterial endotoxin or other toxins provides another novel method for specific targeted delivery of toxins. In yet another method of targeted delivery of toxins, DNA sequences coding regions of the enzymatically active domain of gelonin, bacterial endotoxin or other toxins, or DNA sequences coding for pro-apoptotic inducers, cell cycle blockers, cell proliferation

inhibitors, and differentiation inducers can be ligated to DNA coding specific scFv or Fab fragments.

In another aspect of the present invention, there is provided a method for producing reagents that direct poisons to target cells but not to non-target cells, comprising: creating a scFv heavy and/or light chain or Fab Ig fragment-expressing phage display library; selecting said phage display library for antibody fragments that bind to target cells but not to non-target cells; engineering scFv or Fab for non-phage display; amplifying scFv or Fab in E. coli; collecting secreted scFv or Fab Ig fragments and attaching said scFv Ig fragments to a toxin.

In yet another aspect of the present invention, there is provided a method of killing a fire ant, comprising the step of contacting said ant with the scFv or Fab polypeptide, such as one produced by the method disclosed herein.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments. These embodiments are given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features,
5 advantages and objects of the invention, as well as others which will
become clear, are attained and can be understood in detail, more
particular descriptions of the invention briefly summarized above
may be had by reference to certain embodiments thereof which are
illustrated in the appended drawings. these drawings form a part of
10 the specification. It is to be noted however, that the appended
drawings illustrate preferred embodiments of the invention and
therefore are not to be considered limiting in their scope.

Figure 1 depicts schematically the methods of the
present invention, including immune priming; cDNA preparation;
15 creation of a phage library; phage selection; verification of specific
scFvs and Fabs; and testing thereof.

Figure 2 shows the immunohistological analyses of
monoclonal antibodies binding to midgut antigens of imported and
native fire ant queens. **Figure 2A** shows the absence of binding of

irrelevant antibody to midgut antigens from imported fire ants and serves as the negative control.

Figure 2B shows the binding of monoclonal antibody to midgut antigens from imported fire ant queens.

5 **Figure 2C** shows the monoclonal antibody does not bind to midgut antigens from native fire ants.

Figure 3 shows the purification of phage-displayed Fab's that bind to midgut antigens of imported fire ant queens. Lanes 1-4 show the western blot analysis of clones of Fab Ig selected for
10 binding to midgut antigens of imported fire ants but not to midgut antigens of native fire ants. Lane C is control.

Figure 4 shows the purification of phage-displayed Fab's that bind to gelonin. Western blot analysis show that clones pComb3/Fab(6) and pComb3/Fab(47) selected for binding to gelonin
15 express the Fab Ig fragments. Lane V shows the bacteria containing virus without Fab Ig. U and IN represent uninduced and induced respectively.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a pest eradication
5 product comprising: a peptide directed against an antigenic epitope
of a gastrointestinal or digestive tract target cell of said pest; and a
toxin. This product will have utility against a wide variety of pests,
including imported fire ant queens, roaches, termites, mosquitoes,
rodents, and birds. Representative toxins which may be used in this
10 product include gelonin, bacterial endotoxin, ribosome inactivating
proteins, pro-apoptotic agents, cell cycle blockers, cell proliferation
inhibitors, and cell differentiation inhibitors. Preferably, the target
cell is a cell in the microvilli of the midgut region of an imported fire
ant. In one embodiment, the peptide is an antibody or antibody
15 fragment specific to said antigen. Representative examples of a
peptide directed against said target cell antigen is an antibody
secreted from hybridoma selected from the group consisting of FA1,
FA4, FA7, FA8, FA9, FA10, FA13, FA14, FA15, and FA17.

The present invention is also directed to a pest
20 eradication product comprising: a peptide directed against an

antigenic epitope of a gastrointestinal or digestive tract target cell of said pest; a peptide directed against an antigenic epitope of a toxin; and a toxin. This product will have utility against a wide variety of pests, including imported fire ant queens, roaches, termites, mosquitoes, rodents, and birds. Representative toxins which may be used in this product include gelonin, bacterial endotoxin, ribosome inactivating proteins, pro-apoptotic agents, cell cycle blockers, cell proliferation inhibitors, and cell differentiation inhibitors.

Preferably, the target cell is a cell in the microvilli of the midgut region of an imported fire ant. In one embodiment, the peptide is an antibody or antibody fragment specific to said antigen.

Representative examples of a peptide directed against said target cell antigen is an antibody secreted from hybridoma selected from the group consisting of FA1, FA4, FA7, FA8, FA9, FA10, FA13, FA14,

FA15, and FA17. Representative examples of a peptide directed against said toxin is an antibody secreted from hybridoma selected from the group consisting of G1, G2, G3, G4, G5, G6, and G7.

Preferably, the peptide directed against said toxin is an antibody fragment derived from phage display library clone selected from the

group consisting of pComb3/Fab(6) and pComb3/Fab(47).

The present invention is also directed to a method of killing a pest, comprising the step of contacting said pest with a pest eradication product disclosed herein.

The present invention is also directed to a peptide
5 directed against a target cell antigen, wherein said peptide is an antibody secreted from hybridoma selected from the group consisting of FA1, FA4, FA7, FA8, FA9, FA10, FA13, FA14, FA15, and FA17.

In another embodiment of the present invention, there is
10 provided a peptide directed against a toxin, wherein said peptide is an antibody secreted from hybridoma selected from the group consisting of G1, G2, G3, G4, G5, G6, and G7.

The present invention is also directed to a peptide
directed against a toxin, wherein said peptide is an antibody
15 fragment derived from phage display library clone selected from the group consisting of pComb3/Fab(6) and pComb3/Fab(47).

The following definitions are given for the purpose of facilitating understanding of the inventions disclosed herein. Any

terms not specifically defined should be interpreted according to the common meaning of the term in the art.

As used herein, the term "monoclonal antibody" or "mAb" refers to an antibody comprised of immunoglobulin heavy and light polypeptide chains with specificity to target cells and is generated and selected from a cloned antibody producing cell.

As used herein, the term "antibody fragment" or "Fab" refers to immunoglobulin based recognition units of minimum size comprised of V-gene segments from immunoglobulin heavy and light chains that exhibit high affinity to target antigens.

As used herein, the "scFv" fragment refers to immunoglobulin based recognition unit of minimum size, a single heavy or light chain, or combined heavy and light chain V-gene Ig fragment (referred to as Fab) with high affinity to target cell.

As used herein, the term "bispecific antibody" refers to either chemically derived or DNA technology derived Fab or scFv immunoglobulin fragments with specificity to two different antigenic determinants, i.e., one arm of the Ig specificity unit reacting with targeted antigen and the other arm reacting specifically with toxins such as gelonin or bacterial endotoxins.

As used herein, the term "phage display library" refers to repertoire of up to 2×10^8 independent clones of immunoglobulin Fab or scFv fragments. Phage displaying fab were screened and selected for specificity to midgut antigenic epitopes of imported fire ant
5 queens.

As used herein the term "toxin" refers to any chemical that behaves in a toxic manner in that it kills cells when introduced into target cells, by being delivered by distinct mechanisms: chemically linked to targeted Ig fragment, bispecific Fab technology,
10 or by DNA technology providing scFv heavy chain-toxin cytotoxic domain. A representative toxin is gelonin, a well-known ribosome inactivating protein or recombinant forms thereof. As used herein, the term "toxin" refers to any chemicals that are "pro-apoptotic", "cell cycle blockers", "cell proliferation inhibitors" and "cell proliferation
15 agents", e.g., cDNA from genes that control cell proliferation, cell cycle, cell differentiation, and cell death.

As used herein, the term "phage displayed Fab" and "phage displayed scFv" refers to a repertoire of Fab or scFv heavy and/or light chain Ig fragments that are displayed on phage and

selected through specificity binding to antigenic epitopes of target cells.

In accordance with the present invention there may be employed conventional molecular biology, immunology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual" (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" (B.D. Hames & S.J. Higgins eds. (1985)); "Transcription and Translation" (B.D. Hames & S.J. Higgins eds. (1984)); "Animal Cell Culture" (R.I. Freshney, ed. (1986)); "Immobilized Cells And Enzymes" (IRL Press, (1986)); B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

A "vector" is a replicon, such as a plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A vector is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient mammal. Such an agent is said to be administered in a "therapeutically effective amount" if the amount

administered is physiologically significant. An agent is physiologically significant if its presence results in a change in the physiology of a recipient mammal. For example, in the treatment of retroviral infection, a compound which decreases the extent of infection or of physiologic damage due to infection, would be considered therapeutically effective.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells, for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A

"cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

Production and screening of monoclonal antibodies with high avidity to specific antigenic epitopes is a well-established and standard laboratory procedure. DNA technology well known to those

having ordinary skill in this art permits the introduction of DNA coding for small immunoglobulin recognition units (called antibody fragments i.e., N-terminal variable domains of heavy and light immunoglobulin chains that exhibit the same antigenic specificity as the intact larger parent antibody) into virus expression vectors (phage) that produce and display the scFv heavy and light chains and combinations of heavy and light chain Ig fragments on their surface (2-9). This technology has been used to specifically target tumor cells for selected destruction; however, to date, this technology has not been applied to specifically target insect pests or other animal pests for destruction.

The phage display method represents a major advance over traditional monoclonal antibodies in that large and diverse repertoires of scFv heavy or light and combinations of heavy and light chain Ig V-region genes can be generated and expressed on the surface of viruses; thereby permitting rapid screening and selection for high-avidity (tight binding) scFv Ig fragments with targeted specificity. Importantly, once specific phage-displayed scFv Ig fragments have been selected for specificity to an antigenic epitope, the DNA that codes for the specific Fab fragment is available for

genetic engineering with DNA coding for enzymatically active domain of gelonin, bacterial endotoxins, or other toxins, programmed cell death (apoptotic) genes as well as genes that disrupt cell proliferation. Producing scFv or Fab Ig fragments with targeted
5 specificity and possess enzymatically active gelonin or bacterial endotoxins or other cell death inducing gene products provides a novel method for targeted delivery of cell death inducing products.

The present invention is directed to a pest eradication product comprising a peptide directed against a gastrointestinal or
10 digestive tract target cell antigen of said pest; and a toxin. In one aspect of this pest eradication product, the peptide is an antibody specific for midgut antigenic epitopes of imported fire ant queens. The target cell antigen, however, may be any antigen in the gastrointestinal or digestive tract of the pest. A representative
15 gastrointestinal or digestive tract antigen is found in the microvilli of the midgut region of an imported fire ant. However, the target specificity is not limited to the mircovilli or imported fire ants, but encompass any cell, tissue or organ of any animal species in which the destruction of such cells or tissues or organs results in the
20 containment or elimination of the animal species. In addition to

specifically targeting imported fire ant queens for destruction, this technology has applications in the selected destruction of all other insect pests such as termites, mosquitoes, and roaches as well as pests of different genera including reptiles, avians and mammals.

5 The pest eradication product may contain various toxins. Representative toxins that disrupt cellular transcription and translation include ribosome inactivating proteins, pro-apoptotic agents, cell cycle blockers, cell proliferation inhibitors, and cell differentiation inhibitors.

10 In one embodiment of the present invention, there is provided a method of killing an imported fire ant, comprising the step of contacting said ant with a fire ant eradication product comprising: a peptide directed against a gastrointestinal or digestive tract target cell antigen of the fire ant; and a toxin. Preferably, the
15 said peptide is an antibody specific to the antigen and the target cell is a cell in the microvilli of the midgut region of an imported fire ant queens. Another method for targeted delivery of toxin involves phage displayed libraries of peptides or proteins that are selected for specificity to midgut antigenic epitopes of imported fire ant queens.

20 Many different toxins can be utilized with the targeted delivery

immunogen preparations from imported fire ant queens. At the completion of the immunizations, spleens were removed. Spleens were cut into two equal sections, one to be used for preparation of hybridomas and the other section to be used for phage displayed Fab fragments. For hybridomas, spleen cells were harvested and fused to the SP2/0-Ag14 myeloma cell line (purchased from ATCC). Following fusion, cells were cultured and selected for hybridomas following standard monoclonal antibody production procedures.

Hybridoma screening Hybridomas were cloned by limiting dilution. Hybridoma supernatants were first screened for the presence of mouse immunoglobulins (IgG or IgM) by ELISA, using rabbit antibodies specific for murine IgG or IgM. Ig-secreting hybridomas identified by ELISA were then tested for the ability of cell supernatants to bind to 5 micron cross sections of imported fire ant midguts as determined by immunohistochemical staining. Hybridomas were next screened for ability to specifically react with 5 micron cryocut cross sections of midgut from imported fire ant queens but not to 5 micron midgut sections from native fire ant queens, using immunohistological analyses. For these studies, frozen

cross sections of midguts from imported fire ant queens and native fire ant queens were air dried and fixed with ice cold acetone. The sections were blocked with normal rabbit serum and then incubated with hybridoma supernatants at room temperature for 30 minutes, and washed three times. Next, the sections were reacted with biotinylated rabbit anti-mouse Ig (rabbit antibodies that detected both murine IgG and IgM immunoglobulins) (VECTASTAIN Elite ABC Kit, Vector Laboratories) for 30 minutes, followed by three washes. The sections were then reacted with VECTASTAIN Elite ABC Reagent and developed with DAB(Vector Labs) using the nickel enhancement. Supernatants from hybridomas of interest were isotyped as to immunoglobulin class and light chain type by ELISA. Hybridomas of interest were frozen and stored in liquid nitrogen for future use, whereas supernatants were stored at minus 70°C for future analysis and affinity purification.

Characterization of hybridomas Analyses of

supernatants from 18 hybridomas by immunohistological analyses revealed 11 monoclonals that were positive for the midgut antigens

of imported fire ant queens and negative to the mid gut antigens of

native fire ant queens, 2 monoclonals that were negative for midgut antigens from both imported and native fire ant queens, and 5 monoclonals that were positive for midgut antigens from both imported and native fire ant queens (Table 1). Isotyping of the Ig's revealed all monoclonals to express kappa light chain type, 12 expressed IgM, 3 expressed IgG1, 1 expressed IgG3, and 2 expressed IgG2a. Of the 11 monoclonals that were positive for midgut antigens of imported fire ant queens and negative for midgut antigens of native fire ant queens, 8 were IgM, 2 were IgG1, and 1 was IgG2a. typical data of immunohistological analyses of midgut tissue sections are depicted in Figure 2, showing a positive reaction with midgut tissue from an imported fire ant queen (Figure 2B), a negative reaction with midgut tissue from a native fire ant queen (Figure 2C), and a negative reaction with midgut tissue section from imported fire ant queen treated with irrelevant antibody (Figure 2A).

TABLE 1

Characterization of Hybridoma Clones

5	Clone Number	Clone Designation	Ig Titer (ELISA)	Ig Isotype (H and L)	Reaction with Midgut	
					Imported	Native
10	FA1	1G747C5F7G10	M	IgM, K	Positive	Negative
	FA2	1F11E305C11C10	H	IgM, K	Positive	Positive
	FA3	2A5G5A1B1B2	M	IgM, K	Positive	Positive
	FA4	2A7A2F7C10F8	M	IgM, K	Positive	Negative
	FA5	2E10D10E9F2F1	L	IgG1, K	Positive	Positive
	FA6	2F11FSH4D9G9	L	IgG3, K	Positive	Positive
15	FA7	2H2D11D2F2D2	M	IgG1, K	Positive	Negative
	FA8	2H6C5B8E4Fs	M	IgM, K	Positive	Negative
	FA9	4A7E6D6D5H5	M	IgM, K	Positive	Negative
	FA10	4B11H12G1OD7E10	M	IgM, K	Positive	Negative
	FA11	4E7C6C12F10G11	M	IgG2a, K	Negative	Negative
	FA12	4G5A9A1H11F11	M	IgM, K	Positive	Positive
20	FA13	5A1E3D12B5D1	M	IgG2a, K	Positive	Negative
	FA14	5A7H9C3E1G2	H	IgM, K	Positive	Negative
	FA15	5B6B1D4F3F4	H	IgM, K	Positive	Negative
	FA16	6A8F6C10D11B12	M	IgM, K	Negative	Negative
	FA17	6B384FD4B12	L	IgG1, K	Positive	Negative
25	FA18	6E7C7B10E7C5	M	IgM, K	Negative	Negative

Elisa assay (Ig titre) was used to screen supernatants for expression of immunoglobulins (H, M, and L refer to high, moderate, and low levels of Ig) prior to screening by immunohistological analyses for ability to react with the midgut antigens of imported and native fire ants. Data is recorded as positive or negative (negative reactions

included no detectable reaction to an extremely low level of reaction). Mouse isotype as to heavy and light chain was determined.

5

Affinity purification of IgG and IgM Cultures of

hybridomas which had been stored in liquid nitrogen were established in standard hybridoma medium. After the cultures were

10 well established the cultures were weaned onto Protein Free Hybridoma Medium (PFHM - Life Technologies, Gaithersburg, MD), to eliminate serum in the culture. Cells for production of antibody were allowed to overgrow for 3 days at which time the supernatant (growth media) was collected, quick frozen in a dry ice/ethanol bath and stored at -70°C for subsequent purification. To purify, 15 supernatants were thawed and concentrated using a Centricon 3 (MWCO 3000) at 4°C. The concentrated samples were then purified using either an Immunopure (A/G) IgG Purification Kit or an Immunopure IgM Purification Kit (Pierce, Rockford, IL) following the 20 protocols included with the kit. The selected antibodies were then desalted. IgG's were desalted using the columns that were included

with the (A/G) IgG kit, and the IgM's were desalted using D-salt Dextran Desalting columns (Pierce). Once purified the IgG's were aliquoted and stored at -20°C and the IgM's were brought to 50% glycerol and stored at -20°C.

5

EXAMPLE 2

Production, screening, and testing of phage displayed Ig fragments to
10 midgut antigens of imported fire ants

cDNA synthesis RNA was isolated from mouse spleens (1/2 spleen from mice immunized with midgut preparations from imported fire ant queens as described in Example 1) using the guanidium isothiocyanate method. cDNA was prepared from 5
15 micrograms of RNA with oligo (dT)₁₆ as a primer. Reverse transcriptase, nucleotides, and buffers were purchased from PERKIN ELMER (RNA PCR Kit, Branchburg, New Jersey) and were used according to the instructions provided by the manufacturer. Fd and L chain cDNA were amplified by PCR. The 5' primers used were
20 Light chain (GTGCCAGATGTGAGCTCGTGATGACCCAGTCTCCA), V heavy

chain a (AGGTCCAGCTGCTCGAGTCTGG), V Hb
 (AGGTCCAGCTGCTCGAGTCAGG), V heavy chain c
 (AGGTCCAGCTTCTCGAGTCTGG), and V heavy chain D
 (AGGTCCAGCTTCTCGAGTCAGG) which introduced restriction sites (Sac
 5 I for light chains and XHO 1 for heavy chains) that facilitate their
 directional cloning into pComb 3. The 3' primers used were k chain
 (TCCTTCTAGATTACTAACAACCTCTCCCCTGTTGAA), C heavy 1
 (AGGCTTACTAGTACAATCCCTGGGCACAAT), thereby the k chain
 primer introduced an Xba 1 site and the heavy chain primer
 10 introduced a Spe 1 site. General conditions for PCR were Taq
 polymerase (Perkin Elmer, Branchburg, New Jersey) at 2.5 U/100-
 microliter reaction mixtures, 200 micromolar deoxynucleoside
 triphosphates, 1 millimolar $MgCl_2$, 5 microliters of cDNA per 100
 microliters of reaction mixture, 150 ng of 5' primer and 150 ng of 3'
 15 primer in 1x buffer as supplied by the manufacturer (Perkin Elmer).
 Reaction mixtures were cycled at 94°C for 1.5 minutes, 52°C for 2.5
 minutes, and 72°C for 3 minutes for a total of 40 cycles. These
 conditions have generated products of the correct size (660 bp) on all
 samples.

Phage display library construction

The M13 phage

surface display vector pComb3 was provided by The Scripps Research Institute, LaJolla, California. The pComb3 vector and light chain PCR fragments were digested with Sac I and Xba I (Boehringer

5 Mannheim, Indianapolis, IN) for three hours. The restricted DNA

were purified by electroelution from agarose gels after electrophoresis. Vector and light chain inserts were ligated at

approximately 1:3 molar ratio with T4 DNA ligase (Stratagene, LaJolla, CA) overnight at 4°C. After ligation, 300 microliters of E. coli

10 XL1-Blue was transformed by electroporation with 5 microliters of ligation mixture. The light chain library was propagated in bulk as

an overnight culture in super broth medium (SB; 30 g of tryptone, 20 g of yeast extract, 10 grams of MOPS per liter, pH 7.0) supplemented

with tetracycline at 10 micrograms/ml and carbenicillin at 50

15 micrograms/ml. Phagemid DNA which contained light chain inserts was isolated and digested with XhoI and SpeI and gel purified. Fd

PCR fragments were digested and purified in the same manner, and

ligated into the restricted plasmid to produce a combinatorial library

containing both Fd and L chain genes. After transformation, 3 mls of

20 SOC medium (Molecular Cloning, Cold Spring Harbor Laboratory

Press, 1989) was added, and the culture was shaken at 220 rpm for 1 hour at 37°C, after which 10 mls of SP medium containing tetracycline at 10 micrograms/ml and carbenicillin at 20 micrograms/ml was added; the culture was then shaken at 300 rpm for an additional hour. After this culture period carbenicillin was added to a final concentration of 50 micrograms/ml and incubation continued for another hour.

The cells were coinfectd with the replication-deficient helper phage BCSM13 (10^{12} PFU) in 100 ml of SB medium containing 10 micrograms/ml of tetracycline and 50 micrograms/ml of carbenicillin, and the culture was shaken for 2 hours. After this time, kanamycin at 70 micrograms/ml was added, and the culture was incubated at 37°C overnight. Phage were isolated from culture supernatants by 4% (wt/vol) polyethylene glycol 8000 and 3% (wt/vol) NaCl precipitation. Phage pellets were resuspended in TBS (50 mM Tris-HCl, pH 7.5/150 millimolar NaCl) plus 1% BSA.

Selection of phages One hundred imported fire ant

queen midguts were homogenized and resuspended in TBS plus 1%

BSA and incubated for 20 minutes with rotation. Then 0.5 mls of the

above phage/Fab preparation were added and incubated for another 2 hours. After washing 10 times with TBST (0.5% Tween 20), the bound phage were eluted with 0.1 molar HCl (pH 2.2)/1 mg/ml of BSA at room temperature and immediately neutralized with 2 molar Tris base. The eluted phage were infected with 2 ml of fresh XL1-Blue (O.D600 = 1) at room temperature for 15 minutes, then 10 mls of pre warmed SB medium containing tetracycline at 10 micrograms/ml and carbenicillin at 20 micrograms/ml was added; phage preparation, and panning were repeated as described above.

Phage displaying Ig fragments that were selected for ability to react with midgut antigens from imported fire ant queens underwent further panning, utilizing midgut preparations from native fire ant queens in order to enrich phage with specificity to midgut antigens of imported fire ant queens. Phage preparations were permitted to react with homogenized midgut antigen preparations from native fire ants, and supernatant containing phage that failed to react with midgut antigens of native fire ants were collected for further study after the homogenates were pelleted by centrifugation.

Testing of phage transit in live imported fire ant queens

Colonies of imported fire ants were established so that each colony had at least 5 or more queens (each colony was set up from a single mound collection). After establishment of the colonies, soluble M13

5 Phage were placed in glucose water (10% glucose w/v) and the ants were allowed to consume the glucose phage mixture ad libitum. Fire ant queens were collected 24, 48 and 72 hours after the phage solution was introduced. Midguts were isolated and homogenized in

10 PBS, then centrifuged to remove any debris. The resulting mixture was plated at various dilutions in LB top agar containing 40ul of a solution of X-gal (20mg/ml in dimethylformamide) and 4ul of IPTG (200 mg/ml) which was poured onto LB plates. The plates were covered and the top agar was allowed to harden. The plates were then inverted and incubated at 37°C. Colonies were present at 12

15 and 24 hours following plating. Pale blue plaques began to form in as little as 4 hours and fully developed by 8-12 hours. Incubation at 4°C for a few hours helps intensify the color. These data show that phage displaying Fab fragments can be successfully introduced to the midguts of live imported fire ant queens via feeding.

Purification of soluble Ig fragments from phage display

library To prepare Fab in soluble form, pComb3 phagemid DNA containing L chain and Fd genes was restricted with Spe I and Nhe I to remove the M13 gene coding sequence. The digested plasmid (4.7
5 kb) was self-ligated and transferred to XL1-Blue bacteria cells. Individual bacteria colonies were grown for 6 hours at 37°C, then induced with 1 mM IPTG overnight at 30°C with shaking.

Bacteria were harvested by centrifugation and soluble Fab was extracted from the periplasmic space by freezing in a dry
10 ice-ethanol bath for 5 minutes followed by thawing in 37°C water bath (this process was repeated 4 times). The soluble Fab (46 Kd at non-reduced condition) was analyzed by Western Blot as shown in Figure 3. The specific antibody was also analyzed by Immunohistochemical staining using a rabbit antimouse IgG Fab
15 antibody (Cotex Biochemicals) as the primary antibody.

EXAMPLE 3

Production, screening and testing of hybridomas to gelonin

Hybridoma Production Gelonin was purchased from

5 Sigma (St. Louis, MO). Gelonin was solubilized in Phosphate Buffered Saline (PBS) at 200 mg/ml, and then mixed with an equal volume of complete Freund's adjuvant to form an emulsion. Balb/C mice (6 weeks of age or older) received 0.5 ml of above immunogen by an intraperitoneal injection. Three weeks after the intraperitoneal
10 injection the mice were subcutaneously injected with 50 mg of gelonin in PBS only, and this subcutaneous injection was repeated twice over a two weeks time period. At the completion of the immunizations (three days after the last subcutaneous injection), spleens were removed. Spleens were cut into two equal sections, one
15 to be used for preparation of hybridomas and the other section to be used for production of phage displaying Fab fragments. For hybridomas, spleen cells were harvested and fused to the SP2/0-Ag14 myeloma cell line (purchased from ATCC). Following fusion, cells were cultured and selected for hybridomas following standard
20 monoclonal antibody production procedures.

Hybridoma screening Hybridomas were cloned by limiting dilution. Hybridoma supernatants were screened for ability to react with gelonin, using an ELISA assay. Supernatants from hybridomas of interest were isotyped as to immunoglobulin class and light chain type by ELISA. Hybridomas of interest were frozen and stored in liquid nitrogen for future use, whereas supernatants were stored at minus 70°C for future analysis and affinity purification.

Characterization of hybridomas Isotope analyses of supernatants from 7 hybridomas with strong reactivity to gelonin by ELISA revealed that all monoclonals with specificity to gelonin were of the IgG1 class and kappa light chain type (Table 2).

TABLE 2

Isotype Characterization of Supernatants from Hybridomas Secreting Antibodies Specific to Gelonin

Clone #	Original Clone	Mouse Ig Class	Light Chain
Type			
G1	1F2E11E3C2D8	IgG1	Kappa
G2	2C10B2F6B9C6	IgG1	Kappa
G3	2C12F12E4B8C2	IgG1	Kappa
G4	2C12G9A4B6B8	IgG1	Kappa
G5	2E1D9E11F3F3	IgG1	Kappa
G6	3G8D3C10D8D7	IgG1	Kappa
G7	4G12B8D7B10D3	IgG1	Kappa

Affinity purification of IgG Affinity purification of IgG1

from hybridomas secreting antibodies specific for gelonin was performed as described in Example 1.

EXAMPLE 4

Production, screening, and testing of phage displayed Ig fragments to gelonin

5 cDNA synthesis RNA was isolated from mouse spleens (1/2 of spleen from mice immunized with gelonin as described in Example 3) using the guanidium isothiocyanate method. cDNA synthesis and PCR were carried out as described in Example 2.

10 Phage display library construction Phage display library was constructed as described in Example 2.

Selection of phages Phage bearing Fab fragments on their surface were selected by panning on gelonin coated wells.

15 Wells of a microtiter plate were coated overnight at 4°C with 1 mg of gelonin solubilized in PBS (phosphate buffered saline). The wells were washed three times with TBST (0.5% Tween 20), then blocked with TBS plus 3% BSA (bovine serum albumin) at 37°C for 1 hour. Blocking solution was removed, and 50 ul of above phage

20 preparations were added and incubated for an additional 2 hours at

37°C. After washing ten times with TBST, the phage that bound to gelonin were eluted with 0.1 Molar HCl (pH 2.2)/ 1 mg/ml of BSA at room temperature and immediately neutralized with 2 M Tris base. The eluted phage were mixed with 2 mls of fresh XL1-blue bacteria (O.D.₆₀₀=1) at room temperature for 15 minutes, then added to 10 mls of prewarmed SB medium containing tetracycline at 10 mg/ml and carbenicillin at 20 ug/ml and permitted to grow. For enrichment purposes, the phage preparation and panning were repeated as described above.

Purification of soluble Ig fragments from phage display library

Fab in soluble form was prepared as described in Example 2. The soluble Fab (46 Kd at non-reduced condition) was analyzed by Western Blot, and specificity to gelonin was analyzed by ELISA.

Western immunoblot analyses of soluble Fab from two different preparations are depicted in Figure 4A and B.

EXAMPLE 5

Targeted delivery of toxin to midgut of imported fire ant queens

The above selected and purified monoclonal antibodies
5 and phage displayed Fab's to midgut antigens of imported fire ants
and to the toxin gelonin were used to specifically deliver gelonin to
the midgut of imported fire ant queens. The toxin gelonin serves as
the prototype for the targeted delivery of toxins; however, the
monoclonal antibodies and phage displayed Fab fragments with
10 specificity to midgut antigens of imported fire ant queens can be
used for specific delivery of other toxic agents to the midgut of
imported fire ants.

Established technologies (10-12) were used for gelonin
attachment to Ig, Fab, and Fab₂ for the specific delivery of gelonin to
15 the midgut of imported fire ant queens. Before forming Ig/gelonin
complexes, tests were performed to determine if monoclonal
antibodies, Fab, and Fab₂ fragments with specificity to midgut
antigens possess the ability to kill imported fire ant queens in the
absence of toxin. Gelonin can be conjugated via a stable thioether

linkage to purified monoclonal antibodies, to purified Fab fragments generated by papain or pepsin digestion, or to scFv fragments generated from phage display library. Fab fragments with specificity to the midgut antigens of imported fire ants can also be conjugated via a stable thioether linkage to antibody fragments with specificity to gelonin to generate bispecific antibodies. Furthermore, polypeptides that bind specifically to midgut antigens of imported fire ant queens and contain an enzymatically active domain of a toxin can be generated by DNA technology and genetic engineering technologies (13, 14).

The following references were cited herein:

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Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to

which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

5 One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods, procedures, treatments, molecules, and specific
10 compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the
15 claims.

WHAT IS CLAIMED IS:

1. A pest eradication product comprising:

a peptide directed against an antigenic epitope of a

5 gastrointestinal or digestive tract target cell of said pest; and

a toxin.

2. The pest eradication product of claim 1, wherein

10 said pest is selected from the group consisting of imported fire ant queens, roaches, termites, mosquitoes, rodents, and birds.

3. The pest eradication product of claim 1, wherein

15 said toxin is selected from the group consisting of gelonin, bacterial endotoxin, ribosome inactivating proteins, pro-apoptotic agents, cell cycle blockers, cell proliferation inhibitors, and cell differentiation inhibitors.

4. The pest eradication product of claim 1, wherein said target cell is a cell in the microvilli of the midgut region of an imported fire ant.

5

5. The pest eradication product of claim 1, wherein said peptide is an antibody or antibody fragment specific to said antigen.

10

6. The pest eradication product of claim 1, wherein said peptide directed against said target cell antigen is an antibody secreted from hybridoma selected from the group consisting of FA1, FA4, FA7, FA8, FA9, FA10, FA13, FA14, FA15, and FA17.

15

7. A pest eradication product comprising:

a peptide directed against an antigenic epitope of a gastrointestinal or digestive tract target cell of said pest;

a peptide directed against an antigenic epitope of a toxin;

and

a toxin.

5

8. The pest eradication product of claim 7, wherein said pest is selected from the group consisting of imported fire ant queens, roaches, termites, mosquitoes, rodents, and birds.

10

9. The pest eradication product of claim 7, wherein said toxin is selected from the group consisting of gelonin, bacterial endotoxin, ribosome inactivating proteins, pro-apoptotic agents, cell cycle blockers, cell proliferation inhibitors, and cell differentiation inhibitors.

15

10. The pest eradication product of claim 7, wherein said target cell is a cell in the microvilli of the midgut region of an imported fire ant.

20

11. The pest eradication product of claim 7, wherein said peptide is an antibody or antibody fragment specific to said antigen or said toxin.

5

12. The pest eradication product of claim 7, wherein said peptide directed against said target cell antigen is an antibody secreted from hybridoma selected from the group consisting of FA1, FA4, FA7, FA8, FA9, FA10, FA13, FA14, FA15, and FA17.

10

13. The pest eradication product of claim 7, wherein said peptide directed against said toxin is an antibody secreted from hybridoma selected from the group consisting of G1, G2, G3, G4, G5, G6, and G7.

15

14. The pest eradication product of claim 7, wherein said peptide directed against said toxin is an antibody fragment

20

derived from phage display library clone selected from the group consisting of pComb3/Fab(6) and pComb3/Fab(47).

5 15. A method of killing a pest, comprising the step of contacting said pest with the pest eradication product of claim 1.

10 16. A method of killing a pest, comprising the step of contacting said pest with the pest eradication product of claim 7.

15 17. A peptide directed against a target cell antigen, wherein said peptide is an antibody secreted from hybridoma selected from the group consisting of FA1, FA4, FA7, FA8, FA9, FA10, FA13, FA14, FA15, and FA17.

18. A peptide directed against a toxin, wherein said peptide is an antibody secreted from hybridoma selected from the group consisting of G1, G2, G3, G4, G5, G6, and G7.

5

19. A peptide directed against a toxin, wherein said peptide is an antibody fragment derived from phage display library clone selected from the group consisting of pComb3/Fab(6) and pComb3/Fab(47).

ABSTRACT OF THE DISCLOSURE

The present invention is drawn to a safe, cost-effective, environmentally-friendly and ecologically-sound bioengineered pest eradication product and uses thereof. Immunological and genetic engineering techniques are used to generate monoclonal antibodies as well as viruses (phage) that display scFv heavy and/or light chain Ig fragments which exhibit high-avidity specific binding to cells of the microvilli of the midgut of imported fire ant queens. The specific monoclonal antibodies and phage displayed antibody Fab fragments are conjugated to a toxin for targeted delivery and destruction of imported fire ant queens, but not native species.

IMMUNE PRIMING
Immunize mice with imported
fire ant midgut tissue

RNA → cDNA
Isolate total RNA from spleen of immunized mice,
prepare cDNA by reverse transcription, amplify
by polymerase chain reaction, purify cDNA from gel

**ANTIBODY LIBRARY ON
SURFACE OF PHAGE**
Create phage display library
expressing 10^6 - 10^8 unique
antibody Fab fragments

DUAL MIDGUT SELECTION
Two-step absorptions to yield
phage displaying antibody fragments
specific for midgut of imported fire ants
and not native fire ants

FINAL MICROVILLI SELECTION
Immunohistochemical verification of
Fab specific to imported fire ant
microvilli cells

TESTING
Test phage/Fab for internalization by
microvilli cells of imported fire ants
when administered by feeding

IMPORTED FIRE ANT ERADICATION
Test phage/Fab/gelonin Conjugate for ability
to selectively kill imported fire ants.

FIGURE 1

Figure 2. Evaluation of Monoclonal Antibodies to Midgut Antigens of Imported and Native Fire Ant queens.

Immunohistological analyses of monoclonal antibody binding to the midgut antigens of imported fire ant queens (Positive, B) but not to the midgut antigens of midgut antigens from native fire ants (Native, C). Midgut antigens from imported fire ants reacted with irrelevant antibody did not stain positive and served as the negative control (Control, A)

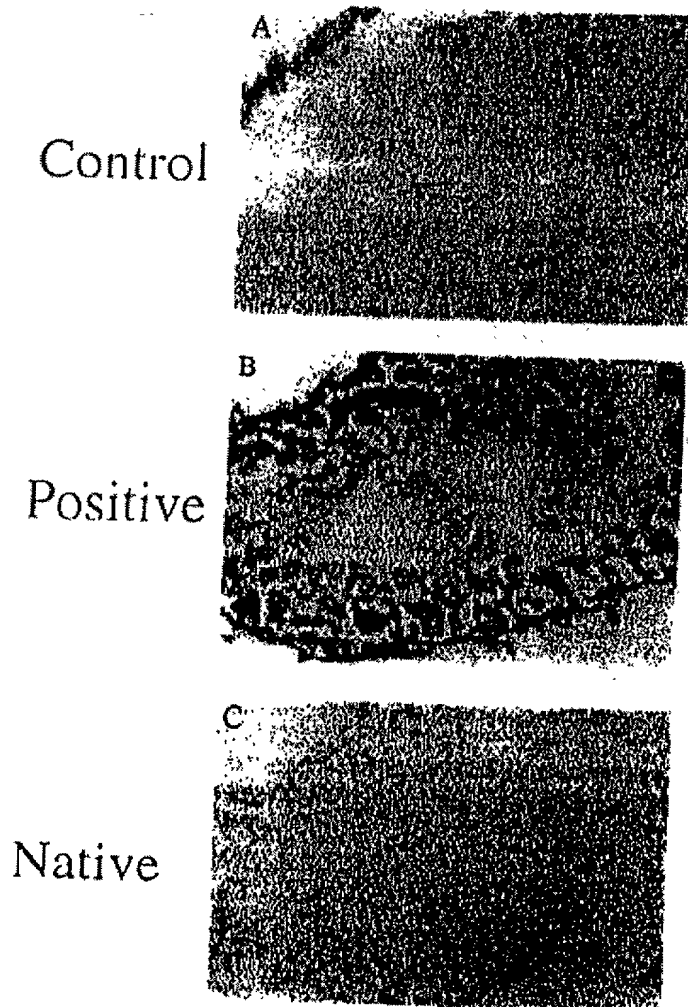


Fig. 2

Figure 2. Purification of Phage Displayed Fab to Midgut Antigens of Imported Fire Ant Queens.

EVIDENCE OF PRESENCE OF IG FAB FRAGMENT

C = Control

1-4 = eluted soluble Fab (sFab)

46 = size of Fab fragment (46 Kb)

Western immunoblot analyses show the clone to express Ig Fab. These clones were selected for ability to bind to antigens of the midgut of imported fire ants but not to the midgut antigens of native fire ants.

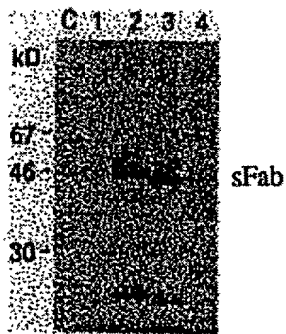


Fig. 3

Figure 4. Purification of Phage Displayed Fab to Gelonin

EVIDENCE OF PRESENCE OF IG FAB FRAGMENT FOR TWO CLONES: (pComb3/Fab (6) and pComb3/Fab (47)).

IN = Induced

U = Uninduced

V = bacteria containing virus without Ig Fab

46 Kb = size of Fab fragment

Western immunoblot analyses show that clones pComb3/Fab (6) and pComb3/Fab (47) express Ig Fab. These clones were selected for ability to bind to gelonin.

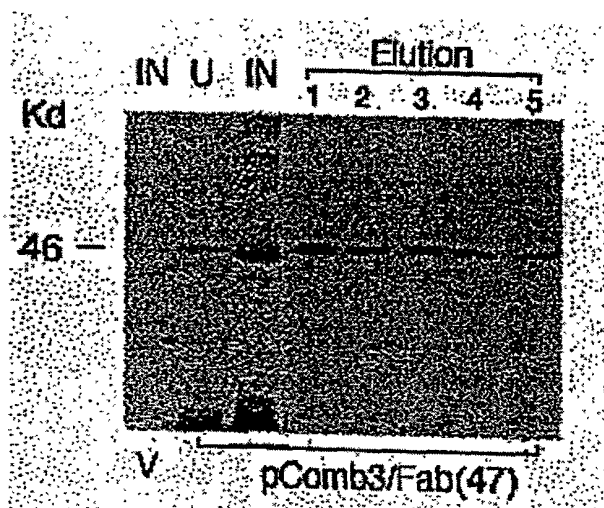
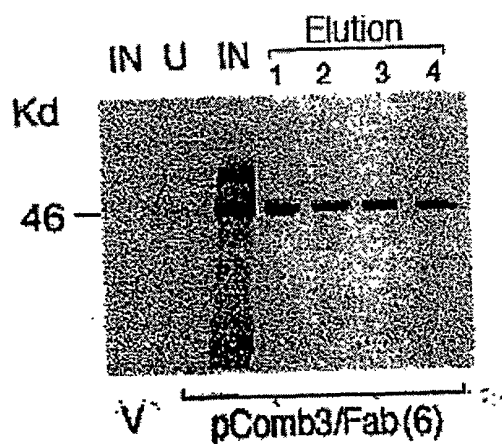


Fig. 4

DOCKET NO: D6017C12

COMBINED DECLARATION AND POWER OF ATTORNEY

I, **Kimberly Kline**, hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled, **Targeted Destruction of Pests**: the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Dr. Benjamin Adler; Registration No. 35,423. Address all telephone calls to Dr. Benjamin Adler at telephone number 713/777-2321. Address correspondence to Dr. Benjamin Adler, MCGREGOR & ADLER, LLP, 8011 Candle Lane, Houston, TX 77071.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patents issued thereon.

Full Name of Inventor: Kimberly Kline

Inventor's Signature: Kimberly Kline Date: 2-9-00

Residence Address: 2505 McCallum, Austin, TX 78703

Citizen of: United States of America

Post Office Address: Austin, TX 78703

DOCKET NO: D6017CI?

COMBINED DECLARATION AND POWER OF ATTORNEY

I, **Robert Sanders**, hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled, **Targeted Destruction of Pests**; the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patents issued thereon.

Full Name of Inventor: **Robert Sanders**

Inventor's Signature: *Robert Sanders* Date: 2-9-00

Residence Address: 2005 Barton Hills Dr., Austin, TX 78726

Citizen of: United States of America

Post Office Address: Austin, TX 78726